Quality by Design approach in the development of hydrophilic interaction liquid chromatographic method for the analysis of iohexol and its impurities

The objective of this research paper is to develop a hydrophilic interaction liquid chromatographic method for the analysis of iohexol, its endo-isomer and three impurities following Quality by Design approach. Design of experiments (DoE) methodology is implemented for the creation of the relationship between critical process parameters and critical quality attributes.

The factors (independent variables) examined are: X_1 = acetonitrile content in the mobile phase (%), X_2 = pH of the water phase and X_3 = ammonium acetate concentration in the water phase (mmol/L). All the factors are continuous. The responses (dependent variables) examined are: k_1 = retention time of related compound C, k_2 = retention time of related compound B, k_3 = retention time of related compound A, k_4 = retention time of exo-iohexol and k_5 = retention time of endo-iohexol. The applied DoE method is Box Behnken design.

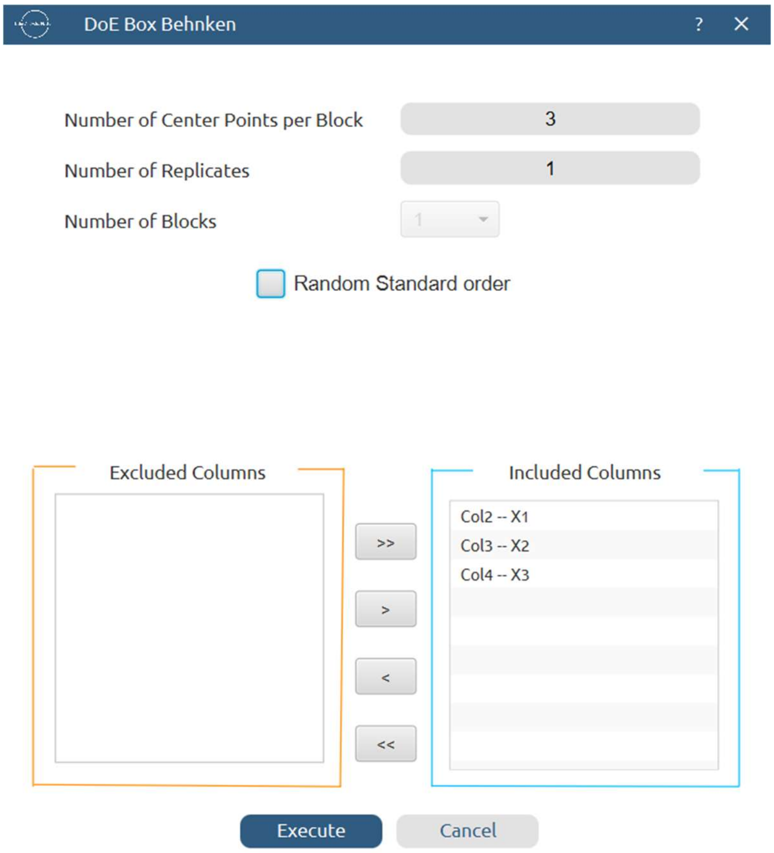
Isalos version used: 2.0.6

Scientific article: https://www.sciencedirect.com/science/article/abs/pii/S073170851500151X

Step 1: Box Behnken Design

In the first tab named "Action" define the factors in the column headers and fill each column with the low and high levels of the corresponding factors. This tab can be renamed "Box Behnken". Afterwards, apply the Box Behnken method: *DOE → Response Surface → Box Behnken*

	Col1	Col2 (I)	Col3 (I)	Col4 (I)
User Header	User Row ID	X1	X2	X3
1		80	3	20
2		90	7	80



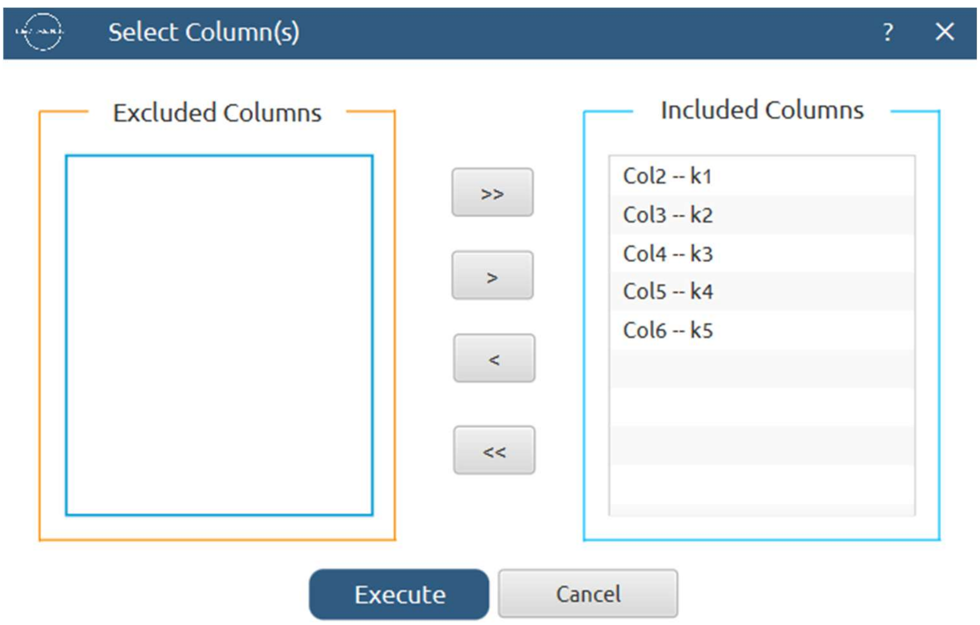
Results (right spreadsheet):

	Col1	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3
1		1	Block: 1	Replicate: 1	Design Point	80.0	3.0	50.0
2		2	Block: 1	Replicate: 1	Design Point	90.0	3.0	50.0
3		3	Block: 1	Replicate: 1	Design Point	80.0	7.0	50.0
4		4	Block: 1	Replicate: 1	Design Point	90.0	7.0	50.0
5		5	Block: 1	Replicate: 1	Design Point	80.0	5.0	20.0
6		6	Block: 1	Replicate: 1	Design Point	90.0	5.0	20.0
7		7	Block: 1	Replicate: 1	Design Point	80.0	5.0	80.0
8		8	Block: 1	Replicate: 1	Design Point	90.0	5.0	80.0
9		9	Block: 1	Replicate: 1	Design Point	85.0	3.0	20.0
10		10	Block: 1	Replicate: 1	Design Point	85.0	7.0	20.0
11		11	Block: 1	Replicate: 1	Design Point	85.0	3.0	80.0
12		12	Block: 1	Replicate: 1	Design Point	85.0	7.0	80.0
13		13	Block: 1	----	Center Point	85.0	5.0	50.0
14		14	Block: 1	----	Center Point	85.0	5.0	50.0
15		15	Block: 1	----	Center Point	85.0	5.0	50.0

Step 2: Definition of response variables

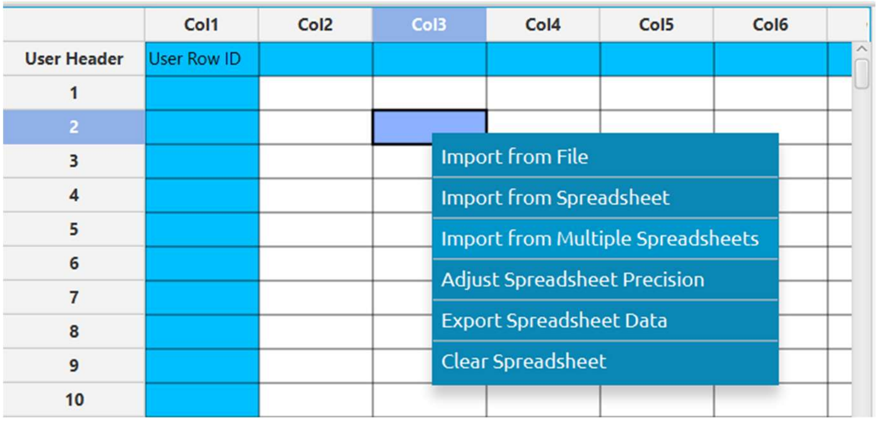
Create a new tab named "Responses" and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Box Behnken method. Then, select all columns to be transferred to the right spreadsheet: *Data Transformation → Data Manipulation → Select Column(s)*

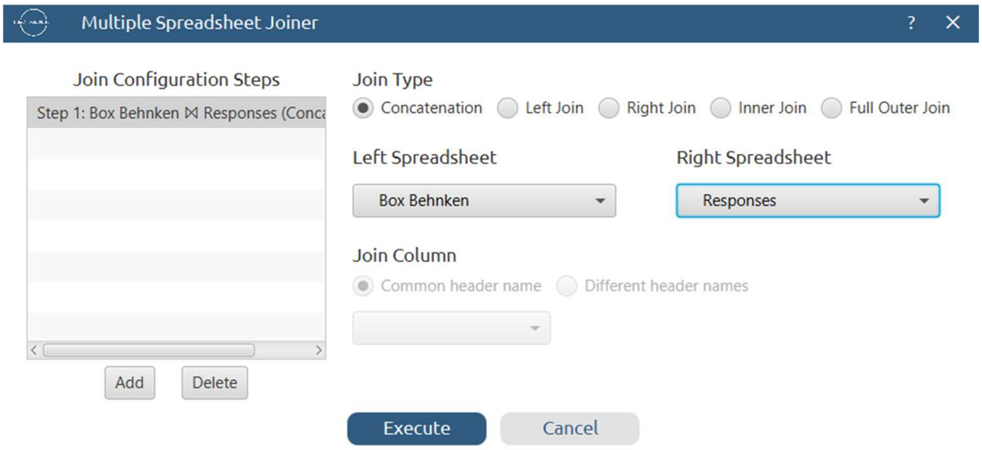
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)
User Header	User Row ID	k1	k2	k3	k4	k5
1		0.11	0.41	0.71	1.11	1.39
2		1.45	3.28	6.16	12.04	15.31
3		0.1	0.38	0.66	1.03	1.29
4		1.3	2.86	5.41	10.31	13.13
5		0.08	0.35	0.61	0.96	1.2
6		1.26	2.8	5.2	9.95	12.51
7		0.11	0.4	0.71	1.12	1.4
8		1.35	2.9	5.62	10.66	13.73
9		0.36	0.93	1.58	2.63	3.27
10		0.37	0.89	1.53	2.55	3.17
11		0.44	1.04	1.83	3.05	3.84
12		0.42	0.96	1.7	2.81	3.53
13		0.38	0.92	1.61	2.66	3.34
14		0.39	0.91	1.61	2.67	3.35
15		0.4	0.92	1.62	2.68	3.18

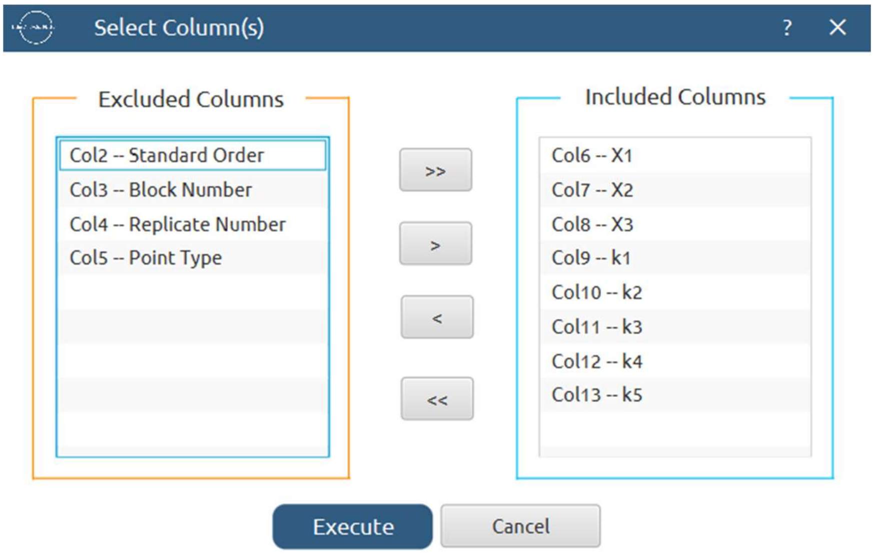


Step 3: Data isolation

Create a new tab named "Data" and import the results from the "Box Behnken" and "Responses" spreadsheets by right clicking on the left spreadsheet. Then, select only the factors and responses columns to be transferred to the right spreadsheet: *Data Transformation → Data Manipulation → Select Column(s)*





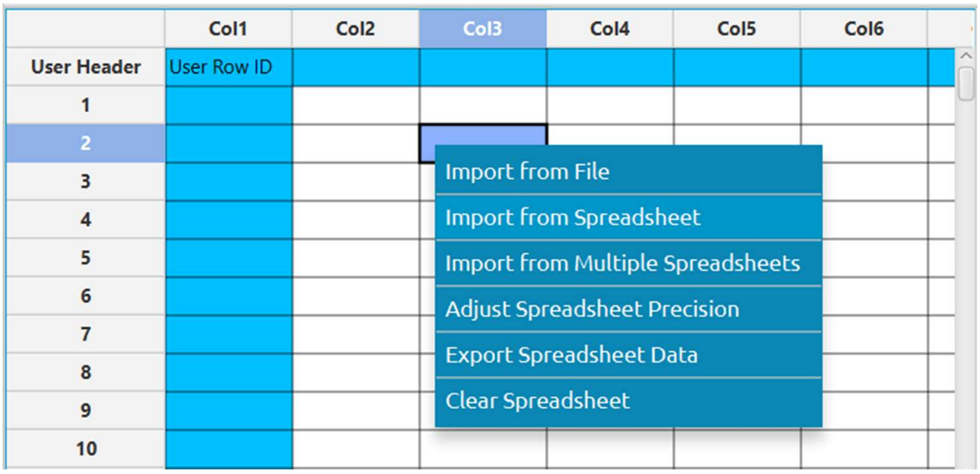


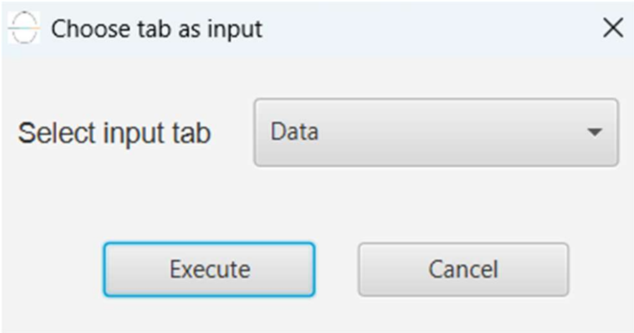
Results:

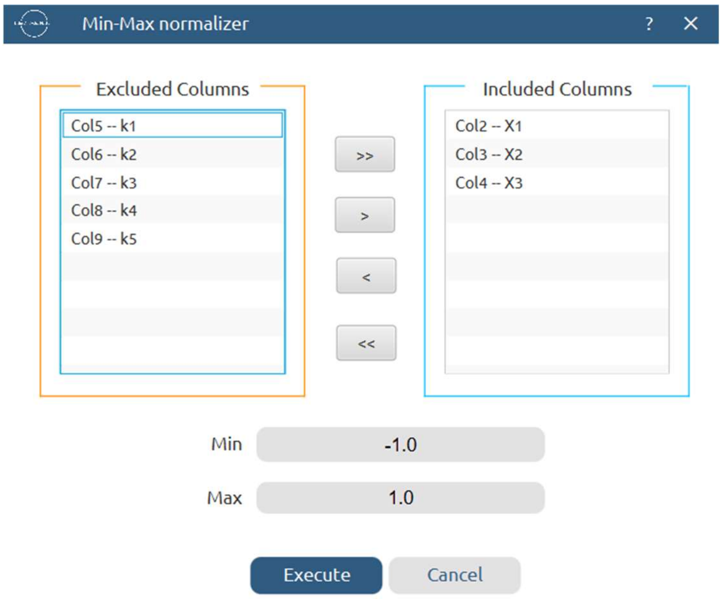
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	k1	k2	k3	k4	k5
1		80.0	3.0	50.0	0.11	0.41	0.71	1.11	1.39
2		90.0	3.0	50.0	1.45	3.28	6.16	12.04	15.31
3		80.0	7.0	50.0	0.1	0.38	0.66	1.03	1.29
4		90.0	7.0	50.0	1.3	2.86	5.41	10.31	13.13
5		80.0	5.0	20.0	0.08	0.35	0.61	0.96	1.2
6		90.0	5.0	20.0	1.26	2.8	5.2	9.95	12.51
7		80.0	5.0	80.0	0.11	0.4	0.71	1.12	1.4
8		90.0	5.0	80.0	1.35	2.9	5.62	10.66	13.73
9		85.0	3.0	20.0	0.36	0.93	1.58	2.63	3.27
10		85.0	7.0	20.0	0.37	0.89	1.53	2.55	3.17
11		85.0	3.0	80.0	0.44	1.04	1.83	3.05	3.84
12		85.0	7.0	80.0	0.42	0.96	1.7	2.81	3.53
13		85.0	5.0	50.0	0.38	0.92	1.61	2.66	3.34
14		85.0	5.0	50.0	0.39	0.91	1.61	2.67	3.35
15		85.0	5.0	50.0	0.4	0.92	1.62	2.68	3.18

Step 4: Normalization

Create a new tab named "Normalized data" and import the results from the "Data" spreadsheet. Afterwards, normalize the factor columns to take values in the range [-1, 1]: *Data Transformation → Normalizers → Min-Max*







Results:

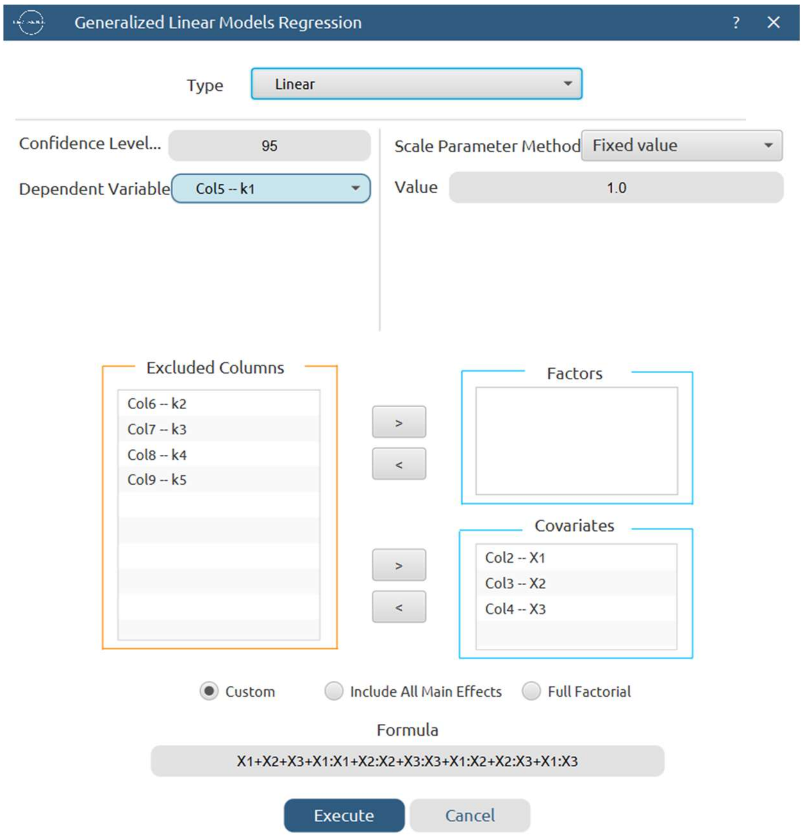
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)
User Header	User Row ID	X1	X2	X3	k1	k2	k3	k4	k5
1		-1.0	-1.0	0.0	0.11	0.41	0.71	1.11	1.39
2		1.0	-1.0	0.0	1.45	3.28	6.16	12.04	15.31
3		-1.0	1.0	0.0	0.1	0.38	0.66	1.03	1.29
4		1.0	1.0	0.0	1.3	2.86	5.41	10.31	13.13
5		-1.0	0.0	-1.0	0.08	0.35	0.61	0.96	1.2
6		1.0	0.0	-1.0	1.26	2.8	5.2	9.95	12.51
7		-1.0	0.0	1.0	0.11	0.4	0.71	1.12	1.4
8		1.0	0.0	1.0	1.35	2.9	5.62	10.66	13.73
9		0.0	-1.0	-1.0	0.36	0.93	1.58	2.63	3.27
10		0.0	1.0	-1.0	0.37	0.89	1.53	2.55	3.17
11		0.0	-1.0	1.0	0.44	1.04	1.83	3.05	3.84
12		0.0	1.0	1.0	0.42	0.96	1.7	2.81	3.53
13		0.0	0.0	0.0	0.38	0.92	1.61	2.66	3.34
14		0.0	0.0	0.0	0.39	0.91	1.61	2.67	3.35
15		0.0	0.0	0.0	0.4	0.92	1.62	2.68	3.18

Step 5: Regression

The goal here is to produce a regression equation that includes main effects, two-factor interactions and quadratic effects for k_1:

$$k = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2$$

Create a new tab named "Regression – k1" and import the results from the spreadsheet "Normalized data". Afterwards, fit a generalized linear model to the data: _Analytics → Regression → Statistical fitting → Generalized Linear Models_



Results:

k1	Prediction
0.11	0.1062500
1.45	1.41625
0.1	0.1337500
1.3	1.3037500
0.08	0.0637500
1.26	1.27375
0.11	0.0962500
1.35	1.3662500
0.36	0.3800000
0.37	0.3525000
0.44	0.4575000
0.42	0.4000000
0.38	0.39
0.39	0.39
0.4	0.39

Goodness of Fit	
	Value
Deviance	0.0048250
Scaled Deviance	0.0048250
Pearson Chi-Square	0.0048250
Scaled Pearson Chi-Square	0.0048250
Log Likelihood	-13.7864905
Akaike's Information Criterion (AIC)	47.5729810
Finite Sample Corrected AIC (AICC)	102.5729810
Bayesian Information Criterion (BIC)	54.6534830
Consistent AIC (CAIC)	64.6534830

#

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	0.39	0.5773503	-0.7415857	1.5215857	0.4563000	1	0.4993583
X1	0.62	0.3535534	-0.0729519	1.3129519	3.0752000	1	0.0794948
X2	-0.0212500	0.3535534	-0.7142019	0.6717019	0.0036125	1	0.9520727
X3	0.0312500	0.3535534	-0.6617019	0.7242019	0.0078125	1	0.9295680
X1*X3	0.0150000	0.5	-0.9649820	0.9949820	0.0009000	1	0.9760671
X1*X2	-0.0350000	0.5	-1.0149820	0.9449820	0.0049000	1	0.9441937
X2*X3	-0.0075000	0.5	-0.9874820	0.9724820	0.0002250	1	0.9880322
X1*X1	0.3262500	0.5204165	-0.6937476	1.3462476	0.3930058	1	0.5307237
X2*X2	0.02375	0.5204165	-0.9962476	1.0437476	0.0020827	1	0.9636000
X3*X3	-0.0162500	0.5204165	-1.0362476	1.0037476	0.0009750	1	0.9750901

Repeat this step for the rest of the response variables. Results, k_2:

k2	Prediction
0.41	0.4187500
3.28	3.1887500
0.38	0.4712500
2.86	2.8512500
0.35	0.2962500
2.8	2.8462500
0.4	0.3537500
2.9	2.9537500
0.93	0.9750000
0.89	0.8525000
1.04	1.0775000
0.96	0.9150000
0.92	0.9166667
0.91	0.9166667
0.92	0.9166667

Goodness of Fit	
	Value
Deviance	0.0337917
Scaled Deviance	0.0337917
Pearson Chi-Square	0.0337917
Scaled Pearson Chi-Square	0.0337917
Log Likelihood	-13.8009738
Akaike's Information Criterion (AIC)	47.6019477
Finite Sample Corrected AIC (AICC)	102.6019477
Bayesian Information Criterion (BIC)	54.6824497
Consistent AIC (CAIC)	64.6824497

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	0.9166667	0.5773503	-0.2149191	2.0482524	2.5208333	1	0.1123512
X1	1.2875000	0.3535534	0.5945481	1.9804519	13.2612500	1	0.0002709
X2	-0.0712500	0.3535534	-0.7642019	0.6217019	0.0406125	1	0.8402877
X3	0.0412500	0.3535534	-0.6517019	0.7342019	0.0136125	1	0.9071195
X1*X3	0.0125000	0.5	-0.9674820	0.9924820	0.0006250	1	0.9800550
X1*X2	-0.0975000	0.5	-1.0774820	0.8824820	0.0380250	1	0.8453929
X2*X3	-0.0100000	0.5	-0.9899820	0.9699820	0.0004000	1	0.9840434
X1*X1	0.7366667	0.5204165	-0.2833309	1.7566643	2.0037333	1	0.1569123
X2*X2	0.0791667	0.5204165	-0.9408309	1.0991643	0.0231410	1	0.8790909
X3*X3	-0.0408333	0.5204165	-1.0608309	0.9791643	0.0061564	1	0.9374599

Results, k_3:

k3	Prediction
0.71	0.7200000
6.16	5.9950000
0.66	0.8250000
5.41	5.4
0.61	0.5350000
5.2	5.3000000
0.71	0.6100000
5.62	5.695
1.58	1.645
1.53	1.4400000
1.83	1.9200000
1.7	1.6350000
1.61	1.6133333
1.61	1.6133333
1.62	1.6133333

Goodness of Fit	
	Value
Deviance	0.1106167
Scaled Deviance	0.1106167
Pearson Chi-Square	0.1106167
Scaled Pearson Chi-Square	0.1106167
Log Likelihood	-13.8393863
Akaike's Information Criterion (AIC)	47.6787727
Finite Sample Corrected AIC (AICC)	102.6787727
Bayesian Information Criterion (BIC)	54.7592747
Consistent AIC (CAIC)	64.7592747

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	1.6133333	0.5773503	0.4817476	2.7449191	7.8085333	1	0.0052000
X1	2.4625000	0.3535534	1.7695481	3.1554519	48.5112500	1	0E-7
X2	-0.1225000	0.3535534	-0.8154519	0.5704519	0.1200500	1	0.7289803
X3	0.1175000	0.3535534	-0.5754519	0.8104519	0.1104500	1	0.7396324
X1*X3	0.0800000	0.5	-0.8999820	1.0599820	0.0256000	1	0.8728811
X1*X2	-0.1750000	0.5	-1.1549820	0.8049820	0.1225000	1	0.7263387
X2*X3	-0.0200000	0.5	-0.9999820	0.9599820	0.0016000	1	0.9680931
X1*X1	1.4983333	0.5204165	0.4783357	2.5183309	8.2892410	1	0.0039881
X2*X2	0.1233333	0.5204165	-0.8966643	1.1433309	0.0561641	1	0.8126648
X3*X3	-0.0766667	0.5204165	-1.0966643	0.9433309	0.0217026	1	0.8828811

Results, k_4:

k4	Prediction
1.11	1.1337500
12.04	11.64375
1.03	1.4262500
10.31	10.2862500
0.96	0.7737500
9.95	10.1837500
1.12	0.8862500
10.66	10.8462500
2.63	2.7925000
2.55	2.3400000
3.05	3.2600000
2.81	2.6475000
2.66	2.6700000
2.67	2.6700000
2.68	2.6700000

Goodness of Fit	
	Value
Deviance	0.6350250
Scaled Deviance	0.6350250
Pearson Chi-Square	0.6350250
Scaled Pearson Chi-Square	0.6350250
Log Likelihood	-14.1015905
Akaike's Information Criterion (AIC)	48.2031810
Finite Sample Corrected AIC (AICC)	103.2031810
Bayesian Information Criterion (BIC)	55.2836830
Consistent AIC (CAIC)	65.2836830

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	2.6700000	0.5773503	1.5384143	3.8015857	21.3867000	1	0.0000038
X1	4.8425000	0.3535534	4.1495481	5.5354519	187.5984500	1	0.0
X2	-0.2662500	0.3535534	-0.9592019	0.4267019	0.5671125	1	0.4514086
X3	0.1937500	0.3535534	-0.4992019	0.8867019	0.3003125	1	0.5836866
X1*X3	0.1375000	0.5	-0.8424820	1.1174820	0.0756250	1	0.7833162
X1*X2	-0.4125000	0.5	-1.3924820	0.5674820	0.6806250	1	0.4093716
X2*X3	-0.0400000	0.5	-1.0199820	0.9399820	0.0064000	1	0.9362373
X1*X1	3.1825	0.5204165	2.1625024	4.2024976	37.3968231	1	0E-7
X2*X2	0.2700000	0.5204165	-0.7499976	1.2899976	0.2691692	1	0.6038896
X3*X3	-0.1800000	0.5204165	-1.1999976	0.8399976	0.1196308	1	0.7294353

Results, k_5:

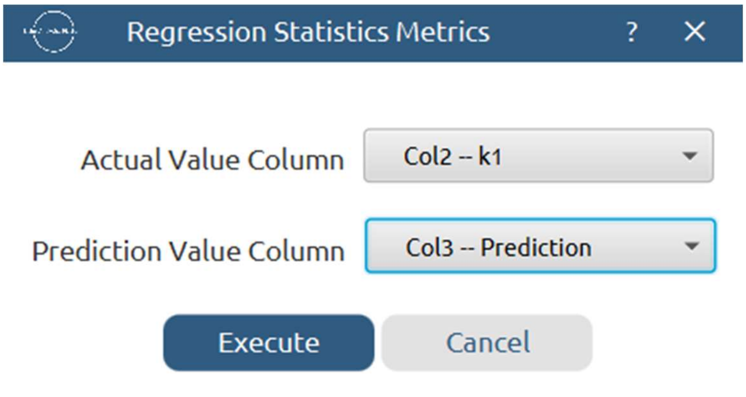
k5	Prediction
1.39	1.4212500
15.31	14.8112500
1.29	1.7887500
13.13	13.0987500
1.2	0.9962500
12.51	12.8362500
1.4	1.0737500
13.73	13.9337500
3.27	3.4425000
3.17	2.875
3.84	4.1350000
3.53	3.3575000
3.34	3.2900000
3.35	3.2900000
3.18	3.2900000

Goodness of Fit	
	Value
Deviance	1.0471250
Scaled Deviance	1.0471250
Pearson Chi-Square	1.0471250
Scaled Pearson Chi-Square	1.0471250
Log Likelihood	-14.3076405
Akaike's Information Criterion (AIC)	48.6152810
Finite Sample Corrected AIC (AICC)	103.6152810
Bayesian Information Criterion (BIC)	55.6957830
Consistent AIC (CAIC)	65.6957830

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	3.2900000	0.5773503	2.1584143	4.4215857	32.4723000	1	0E-7
X1	6.1750000	0.3535534	5.4820481	6.8679519	305.0450000	1	0.0
X2	-0.3362500	0.3535534	-1.0292019	0.3567019	0.9045125	1	0.3415746
X3	0.2937500	0.3535534	-0.3992019	0.9867019	0.6903125	1	0.4060581
X1*X3	0.2550000	0.5	-0.7249820	1.2349820	0.2601000	1	0.6100515
X1*X2	-0.5200000	0.5	-1.4999820	0.4599820	1.0816000	1	0.2983399
X2*X3	-0.0525000	0.5	-1.0324820	0.9274820	0.0110250	1	0.9163758
X1*X1	4.1237500	0.5204165	3.1037524	5.1437476	62.7888519	1	0E-7
X2*X2	0.3662500	0.5204165	-0.6537476	1.3862476	0.4952827	1	0.4815802
X3*X3	-0.2037500	0.5204165	-1.2237476	0.8162476	0.1532827	1	0.6954178

Step 6: Regression Metrics

Create a tab named “Metrics – k1” and import the results from the spreadsheet “Regression – k1”. Then, produce the regression metrics for the k_1 regression equation: *Statistics → Model Metrics → Regression Metrics*



Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0003217	0.0179351	0.0153333	0.9986202

Repeat this step for the rest of the response variables. Results, k_2:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0022528	0.0474634	0.0385556	0.9978101

Results, k_3:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0073744	0.0858746	0.0682222	0.9980739

Results, k_4:

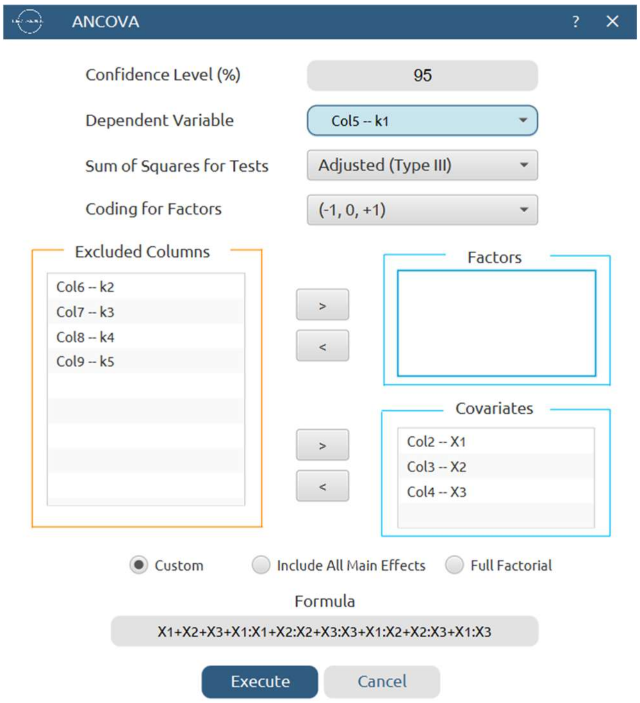
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0423350	0.2057547	0.1630000	0.9972141

Results, k_5:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0698083	0.2642127	0.2183333	0.9971917

Step 7: Analysis of Covariance

Create a new tab named "ANCOVA – k1" and import the results from the spreadsheet "Normalized data". Afterwards perform analysis of covariance for k_1: *Statistics → Analysis of (Co)Variance → ANCOVA*



Results:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	3.0752000	3.0752000	3186.7357513	0E-7
2		X2	1	0.0036125	0.0036125	3.7435233	0.1108040
3		X3	1	0.0078125	0.0078125	8.0958549	0.0360208
4		X1*X1	1	0.3930058	0.3930058	407.2598645	0.0000055
5		X2*X2	1	0.0020827	0.0020827	2.1582304	0.2017501
6		X3*X3	1	0.0009750	0.0009750	1.0103627	0.3609527
7		X1*X2	1	0.0049000	0.0049000	5.0777202	0.0739628
8		X2*X3	1	0.0002250	0.0002250	0.2331606	0.6495899
9		X1*X3	1	0.0009000	0.0009000	0.9326425	0.3785314
10		Error	5	0.0048250	0.0009650		
11		Total	14	3.4968400			

Repeat this step for the rest of the response variables. Results, k_2:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	13.2612500	13.2612500	1962.2071517	1E-7
2		X2	1	0.0406125	0.0406125	6.0092478	0.0578384
3		X3	1	0.0136125	0.0136125	2.0141800	0.2150568
4		X1*X1	1	2.0037333	2.0037333	296.4833539	0.0000121
5		X2*X2	1	0.0231410	0.0231410	3.4240728	0.1234812
6		X3*X3	1	0.0061564	0.0061564	0.9109362	0.3836976
7		X1*X2	1	0.0380250	0.0380250	5.6263872	0.0637942
8		X2*X3	1	0.0004000	0.0004000	0.0591862	0.8174527
9		X1*X3	1	0.0006250	0.0006250	0.0924784	0.7732987
10		Error	5	0.0337917	0.0067583		
11		Total	14	15.4306			

Results, k_3:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	48.5112500	48.5112500	2192.7640500	1E-7
2		X2	1	0.1200500	0.1200500	5.4263975	0.0672562
3		X3	1	0.1104500	0.1104500	4.9924665	0.0757469
4		X1*X1	1	8.2892410	8.2892410	374.6831863	0.0000068
5		X2*X2	1	0.0561641	0.0561641	2.5386817	0.1719677
6		X3*X3	1	0.0217026	0.0217026	0.9809807	0.3674357
7		X1*X2	1	0.1225000	0.1225000	5.5371403	0.0653071
8		X2*X3	1	0.0016000	0.0016000	0.0723218	0.7987297
9		X1*X3	1	0.0256000	0.0256000	1.1571493	0.3312016
10		Error	5	0.1106167	0.0221233		
11		Total	14	57.4298933			

Results, k_4:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	187.5984500	187.5984500	1477.0949963	2E-7
2		X2	1	0.5671125	0.5671125	4.4652770	0.0882720
3		X3	1	0.3003125	0.3003125	2.3645723	0.1847281
4		X1*X1	1	37.3968231	37.3968231	294.4515813	0.0000123
5		X2*X2	1	0.2691692	0.2691692	2.1193593	0.2052169
6		X3*X3	1	0.1196308	0.1196308	0.9419375	0.3763547
7		X1*X2	1	0.6806250	0.6806250	5.3590410	0.0684824
8		X2*X3	1	0.0064000	0.0064000	0.0503917	0.8312681
9		X1*X3	1	0.0756250	0.0756250	0.5954490	0.4751894
10		Error	5	0.6350250	0.1270050		
11		Total	14	227.9421733			

Results, k_5:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	305.0450000	305.0450000	1456.5835024	2E-7
2		X2	1	0.9045125	0.9045125	4.3190283	0.0922696
3		X3	1	0.6903125	0.6903125	3.2962278	0.1291463
4		X1*X1	1	62.7888519	62.7888519	299.8154562	0.0000118
5		X2*X2	1	0.4952827	0.4952827	2.3649645	0.1846978
6		X3*X3	1	0.1532827	0.1532827	0.7319217	0.4313380
7		X1*X2	1	1.0816000	1.0816000	5.1646174	0.0722053
8		X2*X3	1	0.0110250	0.0110250	0.0526441	0.8276149
9		X1*X3	1	0.2601000	0.2601000	1.2419721	0.3157732
10		Error	5	1.0471250	0.2094250		
11		Total	14	372.8643600			

References

(1) Jovanović, M.; Rakić, T.; Tumpa, A.; Jančić Stojanović, B. Quality by Design Approach in the Development of Hydrophilic Interaction Liquid Chromatographic Method for the Analysis of Iohexol and Its Impurities. Journal of Pharmaceutical and Biomedical Analysis 2015, 110, 42–48. https://doi.org/10.1016/j.jpba.2015.02.046.